

pCAR-OF gene



(non-functional b-gal gene: no blue cells)

pCAR-IF gene



(functional b-gal gene: turns cells blue)

FIGURE 1. Engineered genes used to measure the *in vivo* gene altering capability of chemical induced defective mismatch repair. In MMR defective cells, the non-functional β -gal gene is altered to produce a functional protein that can turn cells blue in the presence of X-gal substrate.

H36pCAR-OF Screening Cells

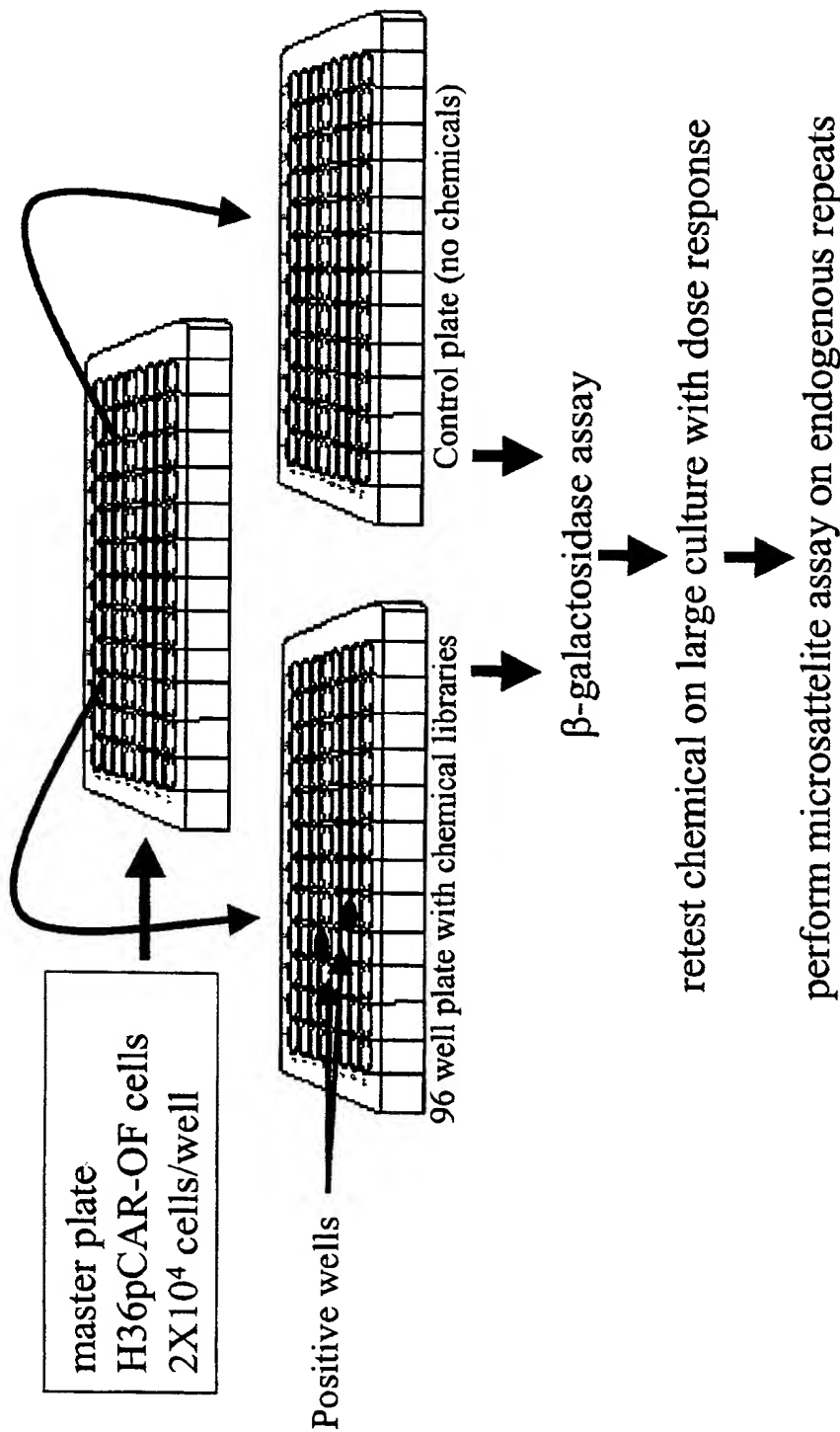


Figure 2: Screening method for identifying mismatch repair blocking chemicals. The assay employs the use of H36pCAR-OF cells which constitutively express the nonfunctional β -galactosidase pCAR-OF gene. Twenty thousand cells are plated in 100 μ ls of growth medium in a 96-well master plate. 50 μ ls of cells (ten thousand cells) are then replated into duplicate wells, one containing chemicals, the other control medium to account for background. Cells are grown for 14 days, lysed and measured for β -galactosidase activity using CPRG substrate buffer. Wells are measured for activity by spectrophotometry at an OD of 576nm. Chemicals producing positive activity are then retested on larger H36pCAR-OF cultures at different doses. Cultures are measured for β -galactosidase and stability of endogenous microsatellite repeats.

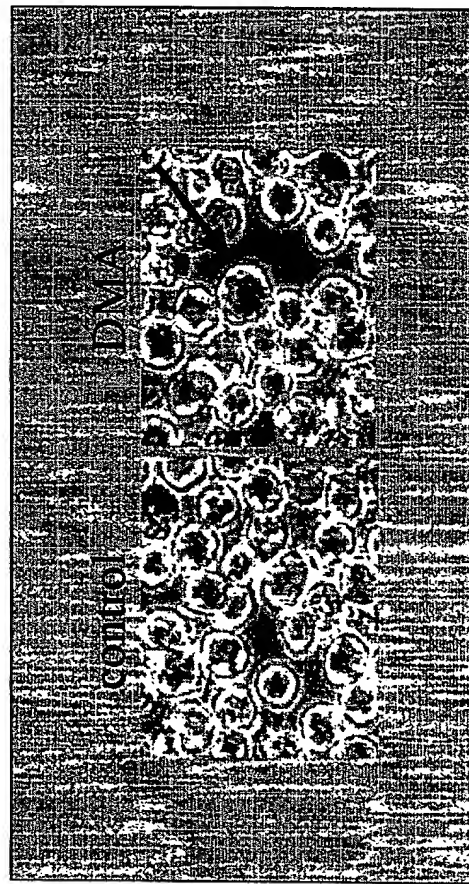


Figure 3. DMA produces b-gal positive H36pCAR-OF cells. H36pCAR-OF cells Grown in the presence of DMA generated functional β -gal producing reporter Cells due to alteration of the polyA repeat within the N-terminus of the construct. The Arrow indicates β -gal positive cells. Approximately 3% of cells were positive for β -gal.

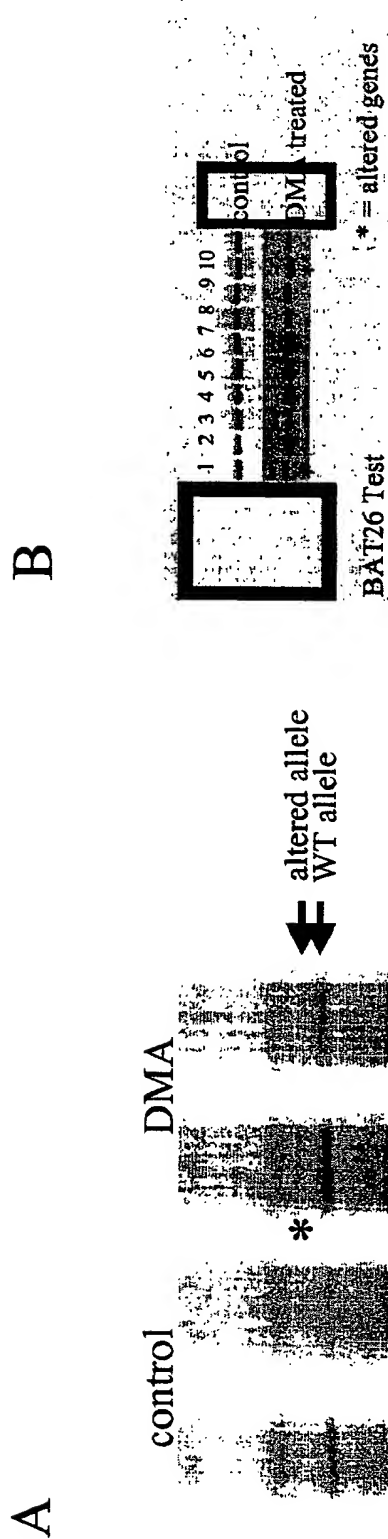


FIGURE 4. Shifting of endogenous microsatellites in human cells induced by DMA in human 293 cells. Cells were cultured in the presence of DMA for 14-17 days. Genomic DNA was isolated and BAT26 microsatellites were analyzed by PCR and gel electrophoresis. (A) Markers were analyzed by PCR using total genomic DNA from 40 samples of treated and untreated cells. Bottom band is the product with the expected wild type (WT) allele size. The asterisk indicates the presence of a new allele in cells treated with DMA. No new alleles were observed in control cells. (B) BAT26 markers from DMA-treated and untreated cells were amplified and cloned into T-tailed vectors. Recombinant clones were then reamplified using BAT26 primers and run on 4% agarose gels and stained with ethidium bromide. Shown is a representative sampling of clones whereby clones with altered molecular weights were observed in DMA treated cells (bottom panel) but not in control cells (top panel). The asterisk indicates markers with altered molecular weight.

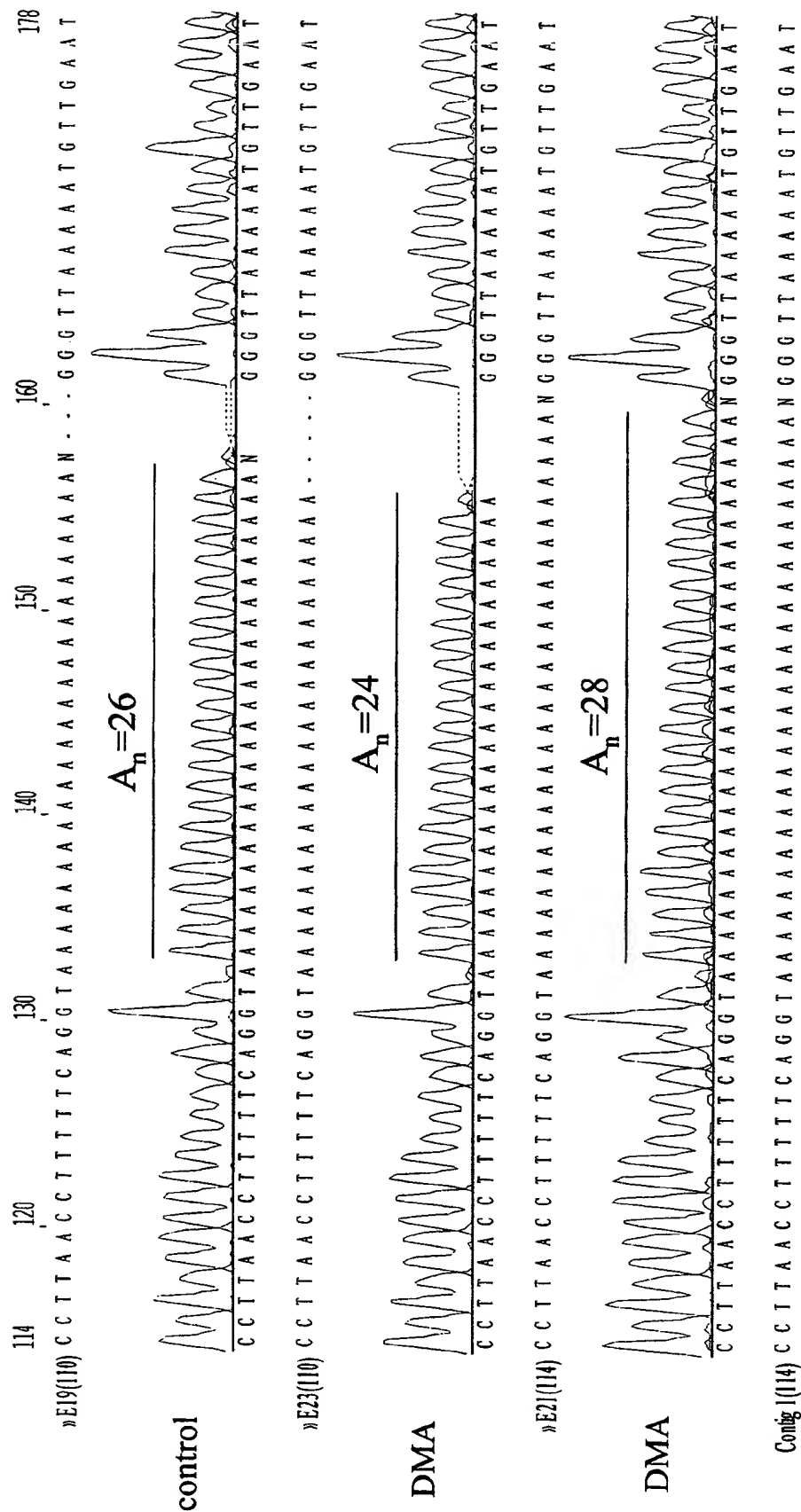
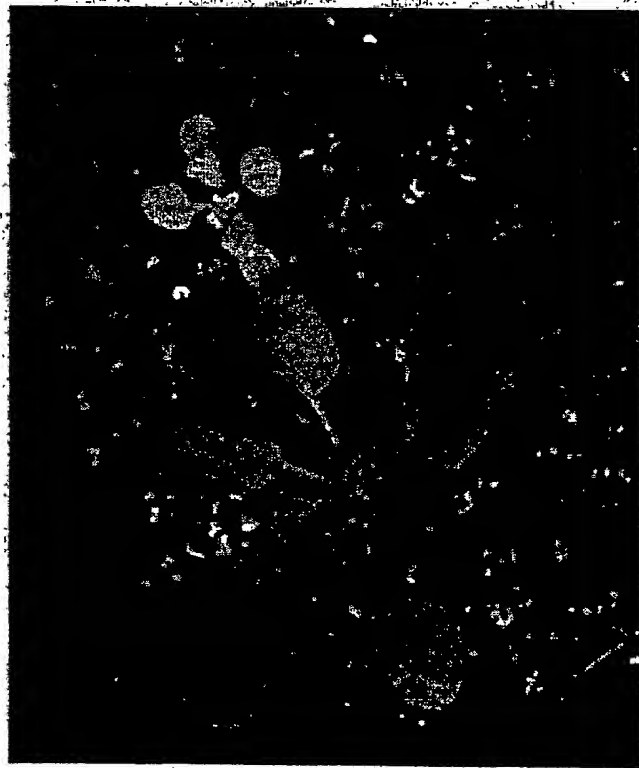


Figure 5. Sequence analysis of recombinant clones containing the BAT26 markers shows alterations within the endogenous polyA repeats in 293 cells treated with 250µm DMA but not in markers obtained from control cells (top sequence). Shown is a sequence alignment from 3 clones. Sequence was aligned using Vector NTI software.



17 day old plants

Figure 6. Chemical inhibitors of MMR blocks spell check process leading to genetic alterations and new output traits. Shown here are offspring from control (WT) or DMA exposed *Arabidopsis thaliana* plants grown in standard soil conditions for 17 days. Six percent of the offspring from DMA treated plants had the small light green appearance. No plants with altered phenotypes were observed in the 150 plants from control or EMS mutagenized offspring. These data demonstrate the ability to generate a high rate of genetic alteration in host organisms by blockade of MMR *in vivo* that can lead to new output traits.

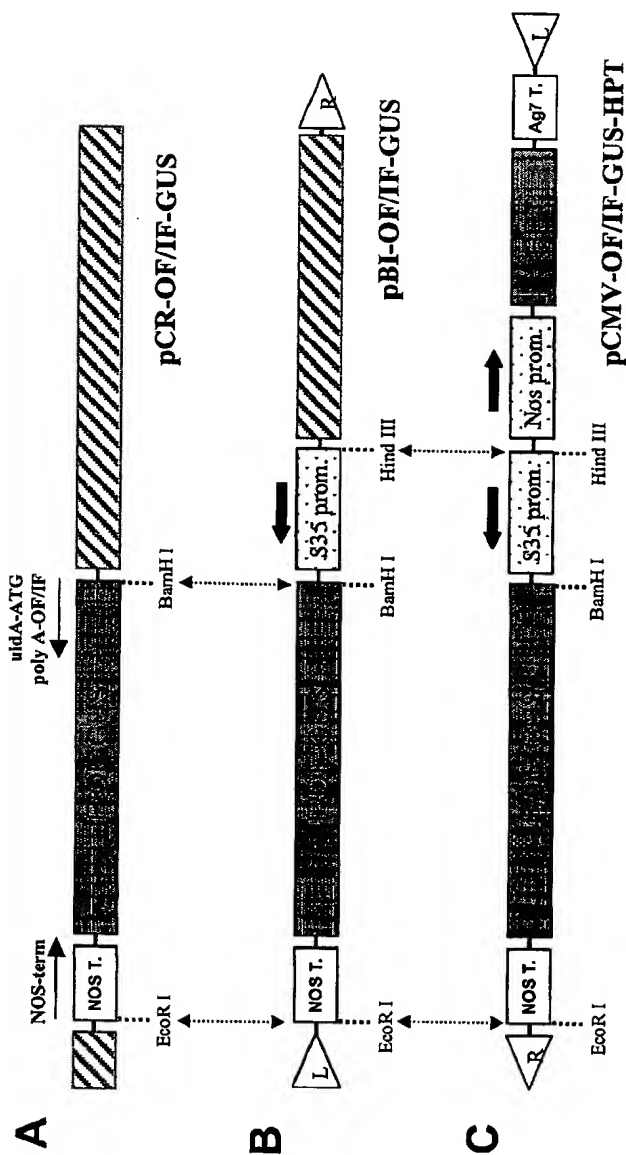


Figure 7. Binary vectors carrying the in-frame (IF) or out-of-frame (OF) version of the β -glucuronidase (GUS) gene. A) IF-GUS and OF-GUS genes, including the nopaline synthase terminator (NOS T.), were obtained by PCR using the NOS-term. and uidA-ATG poly A-OF/IF primers. PCR products were cloned in the TA cloning vector pCR2.1 and sequenced. B) IF-GUS or OF-GUS genes were then cloned into the **EcoRI** and **BamHI** sites of the pBI-121 vector, which carries the Cauliflower Mosaic Virus S35 promoter (S35 prom.). C) The cassette containing the S35 promoter, the IF/OF-GUS gene, and the NOS T. was subsequently cloned into the **EcoRI** and **HindIII** sites of the pGPTV-HPT binary vector, to generate pCMV-IF-GUS-HPT or pCMV-OF-GUS-HPT constructs. HPT, hygromycin phosphotransferase gene. L, T-DNA left border. R, T-DNA right border. Solid arrows indicate direction of transcription. Dotted arrows indicate subcloning sites. Ag7, gene 7 terminator.

Figure 8. Examples of chemical inhibitors of mismatch repair. 9, 10 dimethyl anthracene and anthracene analogs are effective chemical inhibitors of mismatch repair *in vivo*.

